

Arginine vasopressin and adrenocorticotropin secretion in response to psychosocial stress is attenuated by ethanol in sons of alcohol-dependent fathers

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Abstract

Familial risk and environmental stress promote the development of alcohol dependence. We investigated whether a positive family history of alcoholism affects the neuroendocrine response to a standardized laboratory stress test in healthy subjects without alcohol use disorders. Twenty-four high-risk subjects with a paternal history of alcoholism (PHA) and 16 family history negative (FHN) controls were evaluated. Psychosocial stress was induced by having subjects deliver a 5-min speech and mental arithmetics in front of an audience on separate days, after drinking either placebo or ethanol (0.6 g/kg) in a randomized sequence. Adrenocorticotropin (ACTH) was measured in 10 plasma samples covering up to 75 min after the stress test. Plasma arginine vasopressin (AVP) was determined before the stressor, at the time of maximum ACTH secretion, and at 75 min after stress onset. The stress test induced a phasic increase in ACTH secretion. At the time of maximum ACTH, AVP was significantly increased in relation to baseline. Compared to placebo, alcohol administration significantly attenuated maximum ACTH concentration in PHA but not FHN subjects, and decreased AVP measured in the same samples in PHA but not FHN subjects. We conclude that activation of the hypothalamic–pituitary–adrenal system by psychosocial stress is accompanied by an increase in peripheral plasma AVP levels. Secretion of both ACTH and AVP suggest that alcohol attenuates the stress response selectively in PHA but not FHN subjects. This might imply some short-term positive alcohol effect in sons of alcoholics, but also constitute a mechanism by which their risk to develop alcohol use disorders is increased.

Keywords: ACTH; AVP; Psychosocial stress; Alcoholism; Genetic risk; Ethanol; HPA system

1. Introduction

Alcoholism runs in families (Cotton, 1979), and twin studies suggest that a substantial part of the associated risk is conveyed by genetic factors (Prescott and Kendler, 1999). The social environment is also long known to contribute to development and maintenance of alcoholism. Stress appears to be a particularly relevant mechanism (for review, see Sinha, 2001), possibly by interacting with genetic risk factors (Madrid et al., 2001). One possibility to study how the genetic risk manifests itself is to investigate offspring of alcohol-dependent parents who themselves have not yet developed alcohol use disorders (for review, see Newlin and Thomson, 1990). Using this approach, several authors found that offspring of alcoholics had increased autonomic reactivity to aversive electrical shocks and public speaking, and that alcohol had a stronger stress response- dampening effect compared to family history negative controls (Levenson et al., 1987; Finn et al., 1990; Conrod et al., 1998; Sinha et al., 1998).

We were recently able to extend these findings by investigating adrenocorticotropin (ACTH) and cortisol secretion in response to psychosocial stress, which was exaggerated in healthy males with a paternal history of alcoholism (PHA) compared to control subjects with a negative family history (FHN). In this study, alcohol administration significantly attenuated

the endocrine stress response in PHA but not in FHN subjects, while pre-stress hormone levels did not differ between groups (Zimmermann et al., in press). However, while ACTH and cortisol are the core parameters defining a stress response, they provide only indirect information about central nervous control of the hypothalamic–pituitary–adrenal (HPA) system since they are secreted by peripheral endocrine glands. In an effort to complement these data by a parameter which is more closely related to the central nervous processes that elicit the HPA activation, this paper reports on arginine vasopressin (AVP) secretion in a subgroup of the same subjects. Release of ACTH from the anterior pituitary lobe is stimulated by corticotropin releasing hormone (CRH) and AVP. In rodents, the pattern of stress-induced ACTH secretagogue activity depends on the type of stressor. The HPA activation elicited by physical stress is mainly caused by CRH, while other stressors such as novelty, social isolation, restraint, or fear are believed to predominantly result in AVP secretion in animals (Romero and Sapolsky, 1996).

The nonapeptides AVP and oxytocin (OXT) are synthesized in, transported by, and secreted from two distinct classes of neurons. Magnocellular vasopressinergic and oxytocinergic neurons of the hypothalamic paraventricular (PVN), supraoptic, and accessory nuclei constitute the hypothalamic–neurohypophyseal system (Hatton, 1990), whereas parvocellular vasopressinergic neurons are found in the hypothalamus within the suprachiasmatic nucleus (SCN) and the parvocellular part of the PVN (De Vries et al., 1985). The neurons of SCN and PVN project predominantly to the median eminence (Alonso and Assenmacher, 1981), where AVP and CRH are secreted into the portal blood to act synergistically as secretagogues of ACTH at the adenohypophysis (Plotsky, 1991). The parvocellular vasopressinergic system originating from the PVN appears to be activated in depressive syndromes (for review, see Holsboer and Barden, 1996; for animal model see Wigger et al., 2004), which suggests that increased secretion of AVP might contribute to the hyperactivity of the HPA system in major depression. This notion is supported by one study reporting increased plasma AVP in depressive patients compared to healthy controls (van Londen et al., 1997), while other studies found no difference in plasma (Brunner et al., 2002) or cerebrospinal fluid AVP (Heuser et al., 1998; Brunner et al., 2002).

In recent years, evidence has been accumulated that both AVP and CRH act not only as hormones, but also as neuromodulators and neurotransmitters within the central nervous system. Following its central release (Landgraf, 1995) and independent of HPA system regulation, AVP appears to be critically involved in a variety of brain functions including learning, memory, and emotionality (de Wied et al., 1993; Landgraf, 2001; Engelmann et al., 1996).

AVP measured in peripheral blood predominantly originates from the magnocellular system and is secreted in response to osmotic stimuli, its main function being regulation of osmolality (for review, see Lightman, 1990; Hussy et al., 2000). On the other hand, psychological stress activates the parvocellular system to release ACTH secretagogues into the hypophyseal portal vein system (Herman, 1995; for review, see Romero and Sapolsky, 1996). This was associated with an increase in peripheral blood AVP in various animal models (Romero and Sapolsky, 1996), and in humans preparing for their first parachute jump (Dugue et al., 1993). Pain due to injuries was also associated with increased peripheral AVP levels (Kendler et al., 1978), while milder stressors such as noise combined with mental arithmetics and cold stress (Ehrenreich et al., 1997) did not stimulate AVP secretion.

Several studies investigated alcohol effects on peripheral AVP in humans. Oral dosages of 0.25 (Gianoulakis et al., 1997), 0.5 (Chiodera and Coiro, 1990; Gianoulakis et al., 1997), or

0.88 g/kg (Inder et al., 1995) did not alter basal AVP levels, while two studies found a decrease after 0.8 g/kg (Eisenhofer and Johnson, 1982) or approximately 0.4 g/kg (Leppaluoto et al., 1992). Even a low dosage of approximately 0.5 g/kg almost abolished the rise in AVP induced by insulin hypoglycemia (Chiodera and Coiro, 1990). On the other hand, if alcohol induced nausea, this was associated with a marked increase of peripheral AVP and ACTH (Inder et al., 1995).

These observations lead us to investigate plasma AVP levels in response to a standardized laboratory psychosocial stress test that was performed twice in males with a PHA and in FHN controls, once being sober and once being moderately alcohol-intoxicated. We hypothesized that the stress-induced HPA activation might be accompanied by an increase in peripheral blood AVP levels, which might be attenuated by prior alcohol administration. According to our findings on ACTH secretion, we expected the AVP response to be higher in PHA subjects, and the alcohol effect to be more pronounced in PHA than FHN subjects. In an exploratory design, we also investigated whether AVP levels are influenced by potentially confounding factors such as plasma osmolality, individual psychiatric comorbidity, or personality trait variables.

2. Methods

2.1. Subjects

Recruitment of participants based on a prospective longitudinal epidemiological survey investigating a representative sample of 3021 adolescents of the Munich area (Lieb et al., 2000). Before entering the present study, participants of this survey had been screened twice for early developmental stages of psychopathology by means of the Munich Composite International Diagnostic Interview (M-CIDI, Lieb et al., 2000). 115 of 1533 male participants reported that their father, but not their mother had an alcohol problem. For comparison, 62 males who denied parental alcohol problems were selected from the same sample by matching them for age, history of depressive or anxiety disorders, and alcohol use disorders. These subjects were offered participation in the present study during the final epidemiologic interview. If they agreed, both parents were interviewed using the alcohol use disorders section of the M-CIDI. Additional questions were asked concerning whether alcohol had ever caused health or social problems in their parents, brothers or sisters (i.e., the four grandparents and blood-related uncles and aunts of the study participants).

Based on the parental interviews, the inclusion criteria for high-risk subjects were a DSM-IV diagnosis of alcohol dependence in the father and the absence of an alcohol-related disorder in the mother in order to exclude an effect of fetal alcohol syndrome (paternal history of alcoholism, PHA). Control subjects were included if M-CIDI interviews confirmed the absence of a DSM-IV diagnosis of alcohol use disorders in both parents, and both parents denied alcohol problems in their first-grade relatives (family history negative, FHN). General inclusion criteria applying for participants of both groups were social alcohol drinking at least once every month and consenting to abstain from any illegal substance use during the experimental period.

In a high percentage of subjects selected from the epidemiologic survey the inclusion criteria were not fulfilled (Table 1). To increase the sample size, also friends of study participants were considered for investigation. These subjects and their parents underwent the same interviewing procedure as described above to scrutinize inclusion, exclusion, and matching criteria. All subjects were of Caucasian ethnicity. The recruitment process is summarized in

Table 1. During the screening visit a medical history was taken, and a physical examination, routine laboratory tests, and a psychiatric interview were performed. Current active alcohol or substance abuse and dependence were checked by asking the respective CIDI questions. Probands also completed the Beck depression inventory and the sensation seeking scale according to Zuckerman et al. (1980). After complete description of the study, written informed consent was obtained from all subjects.

After the experiments were performed, two subjects were excluded from evaluation due to a positive cannabinoid screen. Data of three others were excluded because, based on post hoc criteria, their ACTH responses were thought abnormal: Two FHN subject had abnormally high maximum ACTH levels (subject 1: 353 and 256 pg/ml during placebo and alcohol experiments, with AVP levels of 2.42 and 1.48 pg/ml, respectively; subject 2: ACTH 87 and 100 pg/ml, AVP 2.1 and 1.8 pg/ml, respectively). One PHA subject showed chaotic decrements and increments of ACTH secretion after the stressor which, contrary to all other subjects, did not define an unambiguous local maximum and questioned the occurrence of a time-organized stress response. Baseline and maximum AVP in this subject were 0.77 and 1.05 pg/ml during placebo, and 0.66 and 0.77 during alcohol. Due to technical problems, AVP could not be measured in the last 12 subjects, altogether leaving 24 PHA and 16 FHN subjects for analysis.

3. Experimental procedures

To investigate reactivity to a psychosocial stressor and its modulation by alcohol, subjects were tested twice using the Trier Social Stress Test (Kirschbaum et al., 1993), which requires probands to deliver a five minutes-speech and five minutes of mental arithmetics in front of three observers unknown to them while being video- and audiotaped. Before the stress tests, placebo or alcohol was administered in a double-blind, placebo-controlled crossover-design with a minimum of 1 week between study days. The experimenter was blind against the risk status of the subjects.

Subjects reported at the laboratory at 1300 h and were asked for recent alcohol use and number of cigarettes smoked today. Recent substance use was checked by urine screening. At 1315 h, an IV line was established and 40 ml/h of 0.9% saline was infused to keep the line open for blood drawing. At 1400 and 1420 h, the beverage was administered divided in two portions and consumed over 5 min, respectively. The total amount of alcohol was 0.6 g/kg of laboratory grade ethanol diluted in ice-cold grapefruit juice to give a concentration of 15% (v/v). During drinking, subjects wore a nose-peg to disguise the smell of alcohol. The same volume of plain grapefruit juice was given on placebo days. At 1420 and 1430 h, basal blood samples were drawn. At 1435 h the subjects were instructed about the test procedures and were given 10 min to prepare for their speech. At 1450 h, three health care professionals walked into the room to act as an audience and the stress test was performed as described by Kirschbaum et al. (1993). Blood samples were taken at the beginning of the preparation time, immediately before, and 10, 20, 30, 45, and 60 min after beginning of the stressor. After the experiment, subjects rated feelings of nausea using a 100 mm visual analog scale. Breath alcohol concentration (BrAC) was measured immediately before and 10, 30, 40, 60, and 80 min after beginning of the stress test using an Alcotest 7410 breath analyzer (Draeger Sicherheitstechnik, Lübeck, Germany). The study protocol was approved by the local ethical committee and the experiments were performed in accordance with the declaration of Helsinki. Subjects were paid for participation.

4. Sample treatment and hormone measurements

Blood samples were drawn in devices pretreated with 8 mg EDTA and aprotinin (2,000 kallikrein-inhibiting units per 7 ml blood, Bayer, Leverkusen, Germany). The blood was kept on ice, spun at 1500g within 60 min, and the plasma frozen at -80 °C. ACTH was measured without extraction by a ¹²⁵I-immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA) with a detection limit of 4 pg/ml. The monoclonal antibody did not cross-react with α -MSH, β -MSH, β -LPH, or β -endorphin. Inter- and intra-assay coefficients of variation at 20 pg/ml were below 8%.

Due to the high technical expenditure, AVP was not analyzed from all samples. To increase the probability of detecting an AVP response to the stressor, we selected in each individual the sample yielding maximum post-stress ACTH level for measurement of AVP. For comparison, AVP was measured from the samples taken immediately before and 75 min after beginning of the stress test. AVP was analyzed after extraction (LiChroprep Si 60; Merck, Germany) by a highly sensitive and selective radioimmunoassay (detection limit 0.1 pg/sample; cross-reactivity of the antiserum with other related peptides, including oxytocin <0.7%). Intra- and inter-assay coefficients of variation were <8% and <13%, respectively. Synthetic AVP (Ferring Pharmaceuticals Ltd., Malmo, Sweden) was used as standard, and iodinated nonapeptide (specific activity 2200 Ci/mmol; NEN Life Science Products, Boston, MA) was used as a tracer. Polyclonal rabbit antibodies raised in our laboratory were used at a dilution of about 1:350,000. The data were not corrected for recovery (80–85% range).

5. Statistical methods

The effects of the within-subjects factor “treatment” (alcohol vs. placebo) and of the between-subjects factors “risk group” (PHA vs. FHN subjects) and “administration sequence” (first test placebo/second alcohol vs. first alcohol/second placebo) on baseline AVP and ACTH levels measured after drinking, but before the stressor were tested by three-factorial multivariate analyses of variance (MANOVA) with repeated measures design. Stress effects on AVP and ACTH secretion were also tested by repeated-measures MANOVAs. Thereby, “stress” (before vs. after the stress test) was an additional within-subjects factor next to the three factors mentioned above.

Breath alcohol concentration was expressed as area under the curve (AUC) of all measurements. Together with the subjects’ characteristics given in Table 2 it was considered one of the secondary variables, which were analyzed for effects of risk group and, where applying, administration sequence by univariate ANOVAs for each variable with a continuous data structure. Variables with nominal data structure were analyzed by Fisher’s exact tests. Subject characteristics that differed significantly between risk groups were corrected for their possible confounding influence on the factor effects by including them as a covariate into supplementary MANOVAs.

In cases of significant interactions in the MANOVAs, the simple effects were analyzed by tests with contrasts to localize exactly the effects of the interacting factors. For all post hoc tests the significance level was corrected according to the Bonferroni procedure in order to keep the type I error less than or equal to 0.05. Associations between the secretion of AVP, ACTH, and cortisol in the various experimental phases were tested by calculating the Pearson correlation coefficients. As a nominal level of statistical significance, $\alpha=0.05$ was accepted.

6. Results

Alcohol administration and the experimental procedures were well tolerated by all subjects. None of the participants reported gastrointestinal discomfort.

7. Breath alcohol concentration

BrAC during the time course of the experiments involving alcohol administration is depicted in Fig. 1. ANOVA with BrAC AUC as dependent variable did not indicate a significant effect of the factors group and administration sequence and no significant factor interaction.

8. Effects on ACTH secretion

Maximum stress-induced ACTH secretion during placebo experiments occurred while the test was still ongoing in three subjects (i.e., at 5 or 15 min), immediately after finishing the test (at 25 min) in 25, and between 35 and 60 min after stress onset in 12 subjects. In experiments involving alcohol administration, maximum ACTH secretion during the respective time intervals occurred in 9, 24, and 7 subjects.

The MANOVA with baseline ACTH levels revealed no significant influence of risk group, treatment, or alcohol administration sequence, and no factor interactions. The repeated-measures MANOVA with ACTH before the test and maximum stress-induced ACTH levels as dependent variables revealed significant effects of stress (Wilks' multivariate test, $F [1, 36] = 30.5$, $p < 0.001$), of treatment ($F [1, 36] = 11.7$, $p = 0.002$), and of their interaction ($F [1, 36] = 9.6$, $p = 0.004$). The third-order interaction between stress, treatment, and risk group also significantly influenced ACTH ($F [1, 36] = 6.9$, $p = 0.013$). There were no significant main effects of group or administration sequence. The source of the third-order interaction was further analyzed for simple effects. Tests with contrasts revealed significantly lower peak ACTH levels after administration of alcohol compared to placebo in PHA ($p < 0.05$, see Fig. 2), but not in FHN subjects. Peak ACTH concentration was marginally higher in PHA than FHN subjects during placebo (tests with contrasts, $p < 0.09$), but not alcohol experiments.

9. Effects on AVP secretion

AVP levels immediately before the stress test were not significantly influenced by risk group, treatment, administration sequence, or their interaction (Fig. 2). The repeated-measures MANOVA with AVP levels before the stressor and AVP at the time of maximum stress-induced ACTH secretion indicated significant effects of stress (Wilks' multivariate test, $F [1, 36] = 19.4$, $p < 0.001$), and of the interactions between group x treatment ($F [1, 36] = 4.9$, $p = 0.033$), and group x treatment x stress ($F [1, 36] = 5.0$, $p = 0.032$). The main effects of group, treatment, and administration sequence were not statistically significant. Analysis of the simple effects underlying the interaction between group and treatment revealed that AVP levels at the time of maximum ACTH secretion were decreased by alcohol administration in PHA ($p < 0.05$, tests with contrasts, Fig. 2), but not FHN subjects, and were significantly lower in PHA compared to FHN subjects after alcohol administration ($p < 0.05$, tests with contrasts), but not in placebo experiments.

The repeated-measures MANOVA comparing AVP concentrations before the stressor and at 75 min revealed no significant effects of stress, group, treatment, or administration sequence, and no factor interactions.

10. Correlation analysis

Pearson correlation coefficients revealed no significant interrelation between AVP and ACTH levels for each of the three time points during either alcohol or placebo experiments.

11. Effects of secondary variables

The subjects' characteristics considered as secondary variables are given in Table 2. Risk group differences were found for sensation seeking scale and urine output during the experiment.

When the sensation seeking scale sum score was included as a covariate into the MANOVA that analyzed AVP levels before stress and at maximum stress-induced ACTH secretion, a significant factor interaction of stress x treatment x sensation seeking was found ($F [1, 35]=5.0, p=0.033$). In this analysis, there was only a marginal effect of stress ($F [1, 35]=3.8, p=0.06$), and the main effects of sensation seeking, treatment, risk group, administration sequence, and of the factor interactions involving risk group were not statistically significant. The sensation seeking scores included as a covariate into the MANOVA analyzing ACTH before and after stress did not alter the main effects described above. Plasma osmolality and urine volume also did not affect AVP levels when included as a covariate and did not alter the influence of the main factors.

12. Discussion

The plasma AVP levels found in this study were within the concentration range usually reported in healthy humans (Eisenhofer and Johnson, 1982; Chiodera and Coiro, 1990; Inder et al., 1997; Ehrenreich et al., 1997; van Londen et al., 1997; Altemus et al., 2001). The psychosocial stress paradigm induced a marked activation of the HPA system, as indicated by a significant difference between baseline and peak ACTH levels. This was accompanied by a small but significant rise of plasma AVP levels at the time of peak ACTH secretion compared to AVP before the stressor. At 50 min after termination of the stress test, AVP concentration was back to baseline.

These findings suggest that acute psychosocial stress induces a short-lived stimulation of AVP secretion into the systemic circulation. The extent of stress-induced AVP stimulation was comparable to that induced by pain (Kendler et al., 1978) or sexual arousal (Murphy et al., 1987), but below that induced by surgery (Moran et al., 1964) or exercise stress (Altemus et al., 2001). Another study employing a multifaceted psychological stress test found no effect on AVP secretion (Ehrenreich et al., 1997). These authors did not measure ACTH or cortisol levels, therefore it cannot be excluded that their rather mild stressor was too weak to stimulate the HPA system.

Comparison between the risk groups revealed a marginally higher ACTH, but equal AVP stress responses in PHA compared to FHN subjects during placebo experiments. In order to interpret this variance, the questions of source and functional relevance of peripheral plasma AVP levels arise. In various paradigms of psychological stress such as restraint, forced swimming, or social defeat, AVP release within the PVN itself was stimulated (Wotjak et al., 1996), and increased AVP gene transcription was found in both parvocellular (Ma et al., 1997) and magnocellular neurons of the PVN (Wotjak et al., 2001). Since AVP in the systemic circulation is generally assumed to originate from the neurohypophysis and thus from magnocellular neurons located in both the hypothalamic PVN and supraoptic nucleus

(SON), the stimulation of this neuron population upon stressor exposure might underlie the increased peripheral AVP levels. In this context it is noteworthy that in animal studies involving social defeat or swim stress, stimulation of AVP release within the PVN was not associated with increased peripheral AVP levels (Wotjak et al., 1996, 1998). If secreted into the portal blood/systemic circulation, AVP can contribute to HPA system stimulation (see Romero and Sapolsky, 1996 for review). In humans, this assumption is supported by findings of a positive correlation between AVP and ACTH levels in depressive patients (Inder et al., 1997) and in healthy individuals after i.v. calcium infusion (Chiodera et al., 1997). In the stress experiments reported here and during HPA stimulation induced by insulin hypoglycemia in obese women (Weaver et al., 1993), such an association could not be observed. These findings question a functional role of peripheral AVP regarding stimulation of the HPA system in our experimental paradigm. Rather, ACTH and AVP might reflect different aspects of the neuroendocrine response to psychosocial stress.

Alcohol administration left baseline ACTH and AVP secretion unaffected and did not alter the neuroendocrine stress response in FHN subjects, but attenuated the stress-induced ACTH and AVP response in PHA subjects. These results support our main hypothesis, namely that alcohol reduces stress-induced HPA activation more effectively in PHA than FHN subjects. This is of particular relevance since AVP is secreted directly from the axon terminals of hypothalamic neurons and thus reflects central nervous reactivity more closely than does anterior pituitary hormone secretion. Together, these findings provide neurobiological evidence for a more effective stress response-dampening by alcohol in healthy, nondependent subjects with a family genetic risk for alcoholism compared to FHN subjects.

Much of the variance in stress-induced AVP secretion was explained by the subjects' sensation seeking scale scores, which were significantly higher in PHA than FHN subjects. This personality dimension is partly determined by genetic factors (Koopmans et al., 1995) and largely overlaps with novelty seeking (McCourt et al., 1993). In children, a high score in novelty seeking is associated with increased rates of adult alcohol abuse (Cloninger et al., 1988), and this trait is part of the concept that distinguishes between type I and type II alcoholism (Cloninger, 1987). In our sample, the sensation seeking sum score correlated negatively with peak AVP levels during alcohol experiments (Pearson correlation coefficient $r = -0.46$, $p = 0.003$) and positively with their difference between placebo and alcohol condition ($r = 0.47$, $p = 0.002$), i.e., higher sensation seeking scores were associated with less AVP secretion during alcohol sessions and a stronger stress dampening effect of alcohol across risk groups. Therefore, it is possible that the same biological factor underlies both the increased sensation seeking and the stronger alcohol effect on stress-induced AVP response in PHA subjects.

A confounding effect of age, weight, recent alcohol drinking, cigarette smoking habits, recent illegal drug use, individual psychiatric history, or depression on AVP secretion could be excluded, since these variables did not differ between risk groups (Table 2). Our data do not offer an explanation for the increased urine output in PHA compared to FHN subjects during placebo experiments, since baseline plasma osmolality, body weight, and AVP levels did not differ between risk groups. Alcohol increased urine output equally between groups in the absence of a main effect of alcohol on AVP levels. Therefore, the alcohol-induced diuresis in this study was probably not due to impaired AVP secretion.

Interpretation of our results is restricted by several limitations. First, AVP was measured only at three time points, which precludes observation of a time course. Therefore it remains to be confirmed whether AVP measured at the time of maximum ACTH secretion adequately

reflects the dynamics of stress-induced changes. Second, blood pressure was taken only during the screening visit and not after the stressor, which would have been helpful to appraise the functional relevance of AVP response differences between risk groups. Third, our efforts to recruit a representative population sample were not completely successful due to the high dropout rate before beginning of the experiments. One subject in the PHA group and six in the FHN group were acquaintances of the initially recruited probands. However, the method of recruitment (representative population sample vs. acquaintances) had no significant influence on ACTH or AVP levels before or after stress.

In conclusion, our findings suggest that alcohol dampens the neuroendocrine response after exposure to a psychosocial stressor only in sons of alcohol-dependent fathers, but not in controls. This is independently indicated by plasma levels of ACTH and AVP. Since stress is detrimental to internal homeostasis and promotes the development of alcohol dependence (Koob and Le Moal, 2001), stronger stress response-dampening by alcohol might be advantageous to sons of alcoholics in the short term, but also constitute a mechanism by which their risk to develop alcohol use disorders is increased.

Table 1
Recruitment of subjects and reasons for dropout

	PHA	FHN
Assessed for eligibility	115	84
Subjects declined to participate	9	5
Parents refused interview	7	3
Paternal alcohol-related disorder not matching inclusion criteria	34	9
Maternal alcohol dependence	4	1
Alcohol-related disorders in second degree relatives not matching inclusion criteria	n.a.	13
Attended screening visit	61	53
Subjects meeting exclusion criteria (refused drinking alcohol or abstaining from illegal drugs, active alcohol abuse, elevated liver enzymes, current mood disorder, history of pancreatitis)	10	7
Withdrawal of consent before first experiment (no more time $n = 10$, refusal of blood drawing $n = 2$, relocated $n = 3$, no reasons given $n = 18$)	15	18
Withdrawal of consent during experiments (phobic reaction during stress test $n = 1$, no more time $n = 1$, no reasons given $n = 5$)	5	2
Excluded for positive cannabinoid screen	2	0
Excluded for abnormal ACTH response	1	2
Technical problems with AVP assay	4	8
Analyzed	24	16

PHA, paternal history of alcoholism; FHN, family history negative; n.a., not applying.

Table 2
Subjects' characteristics (mean and SD)

	Paternal history of alcoholism (PHA, <i>n</i> = 24)	Family history negative (FHN, <i>n</i> = 16)		
Age (year)	19.5 ± 2.4	20.5 ± 2.4		
Body weight (kg)	81.1 ± 24.0	78.4 ± 17.2		
Systolic blood pressure (mm Hg)	127 ± 15.2	132 ± 11.2		
Diastolic blood pressure (mm Hg)	77.4 ± 10.2	77.5 ± 9.7		
Drinks per week	8.5 ± 7.8	4.6 ± 4.2		
Sensation seeking scale sum score ^a	24.6 ± 5.3	20.1 ± 6.3		
Beck depression inventory	3.5 ± 3.9	4.5 ± 7.1		
Regular smokers	14 (58%)	6 (38%)		
Illegal drug use during past 6 months	11 (46%)	5 (31%)		
Prior history of alcohol use disorder	8 (33%)	6 (38%)		
Individual history of affective, anxiety, or somatoform disorder	2 (8%)	2 (13%)		
	Placebo day	Alcohol day	Placebo day	Alcohol day
Plasma osmolality before test (mosm/kg)	291 ± 5.4	294 ± 8.7	293 ± 9.9	295 ± 5.2
Urine output (ml/min) during test ^a	2.41 ± 1.36	3.74 ± 1.88	1.27 ± 0.58	2.26 ± 1.32
Nausea during test (mm on VAS scale) ^b	3.8 ± 6.6	6.8 ± 12.2	1.8 ± 3.0	7.5 ± 12.9
Number of cigarettes smoked before test	2.1 ± 3.0	2.4 ± 0.5	2.3 ± 4.2	1.8 ± 3.1
STAI score before test ^c	35.1 ± 5.1	36.0 ± 5.2	32.8 ± 6.5	35.1 ± 9.5

VAS, 100 mm visual analog scale; STAI, state anxiety inventory (Laux et al., 1981).

^aSignificant effect for risk group (PHA vs. FHN).

^bSignificant effect for treatment (placebo vs. alcohol).

^cSignificant effect for administration sequence (first test placebo-second alcohol vs. vice versa).

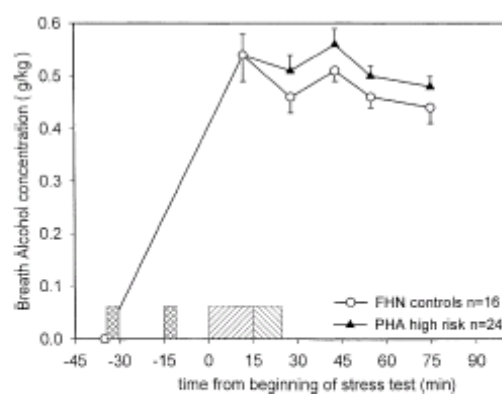


Fig. 1. Mean and SEM of breath alcohol concentration in family history negative control subjects (FHN) and high-risk subjects with paternal history of alcoholism (PHA). Cross-hatched bars: drinking periods, upward hatched bars: preparation time, downward hatched bars: speech and mental arithmetics.

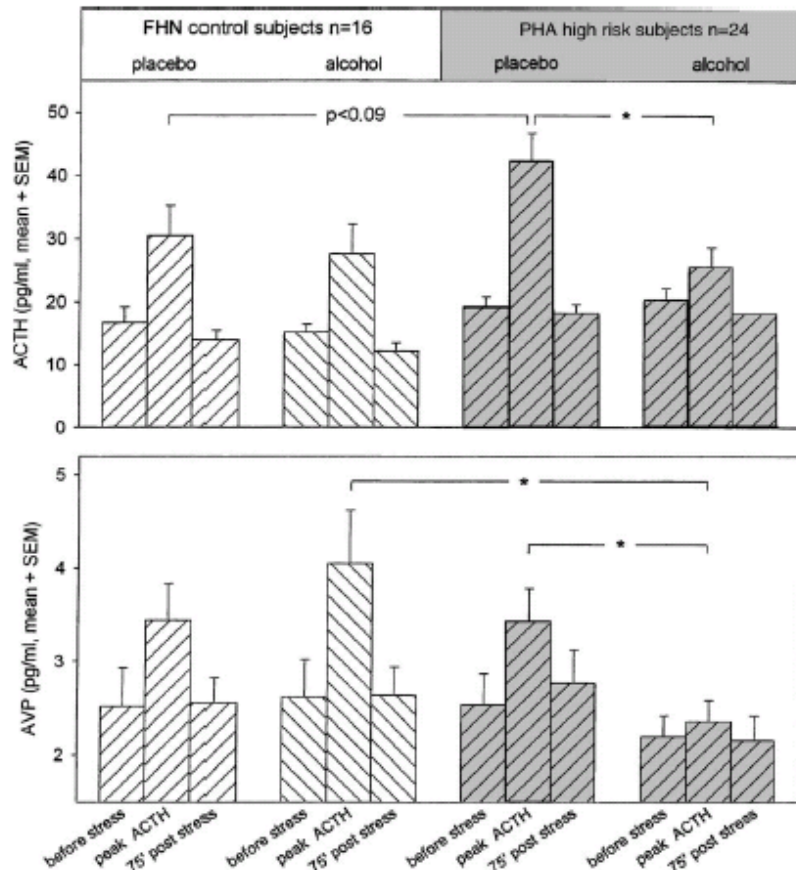


Fig. 2. Plasma ACTH and AVP levels at three selected time points during the stress experiment in FHN controls (white bars) and PHA high-risk subjects (gray bars) after administration of placebo (upward hatched bars) or alcohol (downward hatched bars). * $p < 0.05$, tests with contrasts in MANOVA.

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